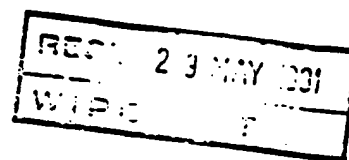




PCT/AU 91 / 00101



Patent Office
Canberra

09/555,442

I, RONALD MAXWELL MAY, ASSISTANT DIRECTOR PATENT OPERATIONS, hereby certify that the annexed is a true copy of the Provisional specification as lodged on 11 February 1991 in connection with Application No. PK 4537 for a patent by BIOTA SCIENTIFIC MANAGEMENT PTY LTD lodged on 11 February 1991.

I further certify that pursuant to the provisions of section 50(1) of the Patents Act 1952 a complete specification was lodged on 24 April 1991 in respect of Applications PJ 9800, PK 2896, PK 4537 and has been allocated No. 75338/91.

I further certify that the annexed specification is not, as yet, open to public inspection.

PRIORITY DOCUMENT

WITNESS my hand this Seventeenth
day of May 1991.

RONALD MAXWELL MAY
ASSISTANT DIRECTOR PATENT OPERATIONS

APPLICANT: BIOTA SCIENTIFIC MANAGEMENT PTY LTD
NUMBER:
FILING DATE:

COMMONWEALTH OF AUSTRALIA

The Patents Act 1952

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

"ANTI-VIRAL COMPOUNDS"

This invention is described in the following statement:

ANTI-VIRAL COMPOUNDS

5 This invention relates to a new class of
anti-viral compounds, including certain 2-deoxy and
2,3-dehydro analogues of α -D-neuraminic acid, and to
their use, via inhibition of viral neuraminidases, for
the prophylaxis and for the treatment of infections such
10 as influenza, Newcastle disease, and fowl plague.

Background of the Invention

Enzymes with the ability to cleave N-acetyl
neuraminic acid (NANA), also known as sialic acid, from
other sugars are present in many microorganisms. These
15 include bacteria such as Vibrio cholerae, Clostridium

perfringens, Streptococcus pneumoniae, and Arthrobacter sialophilus, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of
5 these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such
10 as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

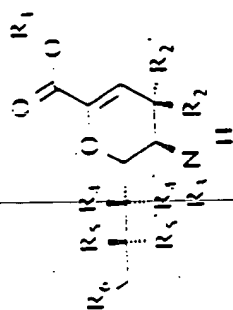
It has long been thought that inhibitors of neuraminidase activity could prevent infection by
15 neuraminidase-bearing viruses. A variety of such inhibitors is known; most are analogues of neuraminic acid, such as 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA) and its derivatives (e.g. Meindl et al, Virology 1974 58 457-63), of which the most active is
20 2-deoxy-2,3-dehydro- N-trifluoracetylneuraminic acid

(FANA). A number of such derivatives is known, and these are summarized in Table 1. FANA inhibits multi-cycle replication of influenza and parainfluenza viruses (Palese et al, Virology 1974 59 490-498). Many of these
25 compounds are active against neuraminidase from V. cholerae or Newcastle disease virus as well as that from influenza virus. Neuraminidase in at least some strains of influenza or parainfluenza viruses is also inhibited by 3-aza-2,3,4-trideoxy-4-oxo-D- arabinooctonic acid δ
30 -lactone and

O- α -N-acetyl-D-neuraminosyl-(2 \rightarrow 3)-2- acetamido-2-deoxy-D-glucose (Zakstel'skaya et al. Vop. Virol. 1972 17 223-228).

TABLE 1

Known 2,3-dehydro derivatives on N-acetylneuraminic acid



	R ₁	R ₂	R ₃	R ₄	R ₅	R ₅	R ₅	R ₆
1	H	H	CH ₃ CO	H	H	H	H	H
2	H	H	NH ₂ CO	H	H	H	H	H
3	H	H	HCO	H	H	H	H	H
4	H	H	FCH ₂ CO	H	H	H	H	H
5	H	H	F ₂ CHCO	H	H	H	H	H
6	H	H	F ₃ CCO	H	H	H	H	H
7	H	H	ClCH ₂ CO	H	H	H	H	H
8	H	H	ICH ₂ CO	H	H	H	H	H
9	H	H	CNCH ₂ CO	H	H	H	H	H
10	H	H	NH ₂ CH ₂ CO	H	H	H	H	H
11	H	H	HSCCH ₂ CO	H	H	H	H	H
12	H	H	CH ₂ CONHCH ₂ CO	H	H	H	H	H
13	H	H	(CH ₃) ₂ NCH ₂ CO	H	H	H	H	H

TABLE 1 (cont.)

	R ₁	R ₂	R ₂ '	R ₃	R ₄	R ₄ '	R ₅	R ₅ '	R ₆
33	H	H	OH	CH ₃ CO-	H	H	OH	H	OH
34	H	H	OH	CH ₃ CO-	OH	OH	H	H	OH
35	H	H	OH	CH ₃ CO-	OH	OH	OH	H	H
36	H	H	H	CH ₃ CO-	H	H	OH	H	OH
37	CH ₃	H	CH ₃ COO-	CH ₃ CO-	H	CH ₃ COO-	CH ₃ COO-	H	CH ₃ COO-
38	CH ₃	H	CH ₃ COO-	CH ₃ CO-	CH ₃ COO-	CH ₃ COO-	H	H	CH ₃ COO-
39	CH ₃	H	CH ₃ COO-	CH ₃ CO-	CH ₃ COO-	CH ₃ COO-	CH ₃ COO-	H	CH ₃ COO-
40	CH ₃	H	H	CH ₃ CO-	H	H	C ₆ H ₅ CH ₂ O-	H	C ₆ H ₅ CH ₂ O-
41	CH ₃	H	C ₆ H ₅ CH ₂ O-	CH ₃ CO-	C ₆ H ₅ CH ₂ O-	C ₆ H ₅ CH ₂ O-	CH ₃ COO-	H	CH ₃ COO-
42	CH ₃	H	CH ₃ COO-	CH ₃ CO-	CH ₃ COO-	CH ₃ COO-	H	H	CH ₃ COO-
43	CH ₃	H	CH ₃ COO-	CH ₃ CO-	CH ₃ COO-	CH ₃ COO-	H	H	CH ₃ COO-
44	CH ₃	H	CH ₃ COO-	CH ₃ CO-	CH ₃ COO-	CH ₃ COO-	H	CH ₃ COO-	H
45	CH ₃	H	CH ₃ COO-	CH ₃ CO-	CH ₃ COO-	CH ₃ COO-	H	CH ₃ COO-	2a-Neu5Ac

TABLE 1 (cont.)

Compounds 33-40	F. Zbrtal et al. Synthesis of 2,7-, 2,8-, and 2,9 Dideoxy and 2,4,7-Tri-deoxy-2,3 didehydro-N-acetylneuraminic Acids and Their Behavior Towards Sialidase from <i>Vibrio cholerae</i> . <i>Enzymes Ann. Chem.</i> 1989, 159-165
Compounds 41-42	F. Ogawa and Y. Ito. An Efficient Approach to Stereoselective Glycosylation of N-Acetylneuraminic Acid: Use of Phenylselenenyl Group as a Stereocontrolling Auxiliary. <i>Tetrahedron Letters</i> 28, (49), 6221-6224(1987).
Compounds 43-45	T. Goto et al. Synthesis of (α 2-9) and (α 2-8) Linked Neuraminylneuraminic Acid Derivatives. <i>Tetrahedron Letters</i> 27, (43), 5229-5232(1986).
Compound 46	H. Ogura et al. Studies on Sialic Acids XV. Synthesis of α and β -Q- Glycosides of 3-Deoxy D-glycero-D-galacto-2-nonulopyranosonic Acid (KDN). <i>Chem. Pharm. Bull.</i> 36, (12), 4807-4813(1988)

Neuraminidase from Arthrobacter sialophilus is inhibited by the glycals 2,3-dehydro-4-epi-N-acetylneuraminic acid, 2,3-dehydro-2-deoxy-N-acetylneuraminic acid, and 5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-mannon-2-en-4-ulosonate, and by their methyl esters (Kumar et al Carbohydrate Res 1981 94 123-130; Kumar et al Carbohydrate Res 1982 103 281-285).

- The thioanalogues 2- α -azido-6-thio-neuraminic acid and 2,3-dehydro-6-thioneuraminic acid (Mack and
10 Brossmer: Tetrahedron Letters 1987 28 191-194) and the fluorinated analogue N-acetyl-2,3-difluoro- α -D-neuraminic acid (Nakajima et al: Agric. Biol. Chem. 1988 52 1209-1215) were reported to inhibit neuraminidase, although the type of neuraminidase was not identified.
15 Schmid et al (Tetrahedron Letters 1958 29 3643-3646) described the synthesis of 2-deoxy-N-acetyl- α -D-neuraminic acid, but did not report its activity or otherwise against neuraminidase.

- Meindl and Tuppy (Hoppe-Seyler's Z. Physiol
20 Chem 1969, 350, 1088) described hydrogenation of the olefinic double bond of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid to produce the β -anomer of 2-deoxy-N-acetylneuraminic acid. This β -anomer did not inhibit Vibrio cholerae neuraminidase.

- 25 While these previously known inhibitors are competitive inhibitors of neuraminidases, none of the compounds is known to have anti-viral activity in vivo. Although Dernick et al (in Antiviral Chemotherapy ed. K.K. Gauri Academic Press 1981 p. 327-336), have asserted
30 that a half-planar, unsaturated 6-member ring system is important for inhibitory activity, some compounds, notably FANA characterized by such a system are reported to have no in vivo anti-viral activity, (Palese, P. and Schulman, J(1977) in Chemoprophylaxis and Virus Infection
35 of the Upper Respiratory Tract Vol 1 CRC Press ed. 1978. Oxford p. 139-205).

Thus conventional wisdom identifies the most potent in vitro inhibitors of viral neuraminidase as compounds that are based on the neuraminic acid framework, and these are thought by some to be transition-state analogues (Miller, C.A., P. Wang, and M. Flashner (1978) Biochem. Biophys. Res. Comm. 83 1479).

We have now surprisingly found that, although FANA was found to be inactive in vivo (Palese and Schulman, op. cit.), DANA has high activity when administered intranasally to mice. It appears that the dose, as well as the route of administration, may be crucial, because the compound is rapidly excreted when given by other routes (Nohle, U., J-M. Beau, and R. Schauer, (1982) Eur. J. Biochem. 126 543-548).

15 Summary of the Invention

It is therefore an object of the present invention to provide improved inhibitors of neuraminidase which have anti-viral activity in vivo.

It is also an object of the present invention to provide medicinal compositions which can be used to prevent or ameliorate symptoms of viral infection.

It is a further object of the present invention to provide means for producing such medicinal compositions.

25 In achieving this object there has been provided, in accordance with one aspect of the invention, a biologically active substance that binds the active site ("receptor") of influenza virus neuraminidase such that said substance displays anti-orthomyxovirus or paramyxovirus activity in an animal. In a preferred embodiment, the active substance displays:

30 (a) in vitro activity in an assay which detects binding of the active site of influenza virus neuraminidase; and

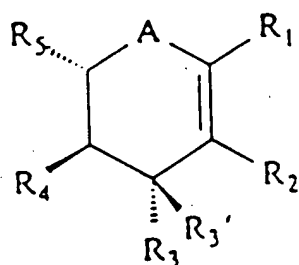
(b) in vivo anti-orthomyxovirus or paramyxovirus activity.

Preferably said in vivo activity is displayed in mice challenged intranasally with influenza virus.

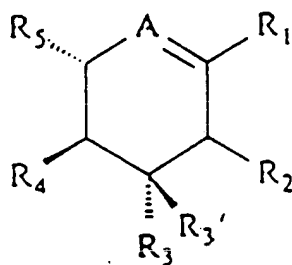
5 According to another aspect, the invention provides a biologically active substance which possesses stereochemical complementarity to an enzyme active site comprised of amino acids positioned at atomic co-ordinates enumerated as part of Figure 1 below, or a
10 subset thereof and said substance displays in vivo activity against an orthomyxovirus or a paramyxovirus. Preferably said stereochemical complementarity is such that said compound has an K_i for said active site of the less than 10^{-7} M. More preferably said K_i is less
15 than 0.5×10^{-8} M.

Preferably according to either aspect the substance is a carbohydrate comprising a non-mutarotatable anomeric carbon atom. More preferably, this carbon atom is optionally substituted by a
20 functional group. Even more preferably, said functional group is carried on the C_2 carbon.

In a preferred embodiment, the compound has general formula I or general formula Ia:



I



Ia

where in general formula I, A is oxygen, carbon or sulphur, and in general formula Ia, A is nitrogen or carbon;

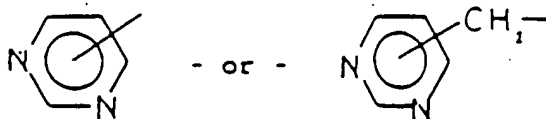
R^1 denotes COOH , $\text{P}(\text{O})(\text{OH})_2$, NO_2 , SOOH , SO_3H , tetrazol, CH_2CHO , CHO , $\text{CH}(\text{CHO})_2$ or, where R^1 is COOH , $\text{P}(\text{O})(\text{OH})_2$, SOOH or SO_3H , an ethyl, methyl or pivaloyl ester thereof,

10 R^2 denotes H, OR^6 , F, Cl, Br, CN, NHR^6 , SR^6 or CH_2X , wherein X is NHR^6 , halogen or OR^6 and

R^6 is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue

thereof; or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, and NO_2 group, and NH_2 group or a COOH group,

R^3 and $\text{R}^{3'}$ are the same or different, and each S denotes hydrogen, CN, NHR^6 , N_3 , SR^6 , $=\text{N-OR}^6$, OR^6 , guanidino,



R^4 denotes NHR^6 , SR^6 , OR^6 , COOR^6 , NO_2 , $\text{C}(\text{R}^6)_3$, CH_2COOR^6 , CH_2NO_2 or CH_2NHR^6 , and

R_5 denotes CH_2YR^6 , $\text{CHYR}^6\text{CH}_2\text{YR}^6$ or $\text{CHYR}^6\text{CHYR}^6\text{CH}_2\text{YR}^6$, where Y is O, NH, S or H, and successive Y moieties in an R^5 group are the same or different,

and pharmaceutically acceptable salts or

derivatives thereof.

15 In both these formulae R^1 , R^2 , R^3 , $\text{R}^{3'}$, R^4 , R^5 and R^6 are subject to the provisos that in general formula I,

(i) when R^3 or $\text{R}^{3'}$ is OR^6 or hydrogen, and A is oxygen or sulphur, then said compound cannot have both

20 (a) an R^2 that is hydrogen and
(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen,

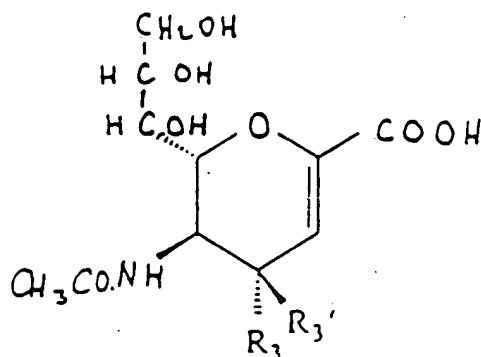
and that in general formula Ia,

25 (i) when R^3 or $\text{R}^{3'}$ is OR^6 or hydrogen, and is nitrogen, then said compound cannot have both

(a) an R^2 that is hydrogen, and
(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen.

In a more preferred embodiment, the compound has general formula II



5 i.e. in general formula I above, R_1 is COOH, R_2 is hydrogen, R_4 is acetamido, and R_5 is $-\text{CHOH}.\text{CHOH}.\text{CH}_2\text{OH}$, and R_3 is hydrogen or R_3' , where R_3' denotes $-\text{N}_3$, $-\text{CN}$, $-\text{CH}_2\text{NH}_2$, or $-\text{N}.\text{R}_8.\text{R}_9$;

R_8 and R_9 are the same or different, and each
10 denotes hydrogen, a linear or cyclic alkyl group of 1 to 6 carbon atoms, an acyl or substituted acyl group of 1 to 6 carbon atoms, $-\text{C}(\text{NH})\text{NH}_2$, $-\text{CH}_2.\text{COOH}$, or $-\text{CH}_2.\text{CH}.$
 $(\text{R}_9)(\text{R}_{10})$,

R_9 and R_{10} may be the same or different, and
15 each denotes oxygen or $\text{R}_{11}\text{N=}$, and
 R_{11} denotes hydrogen, $-\text{OH}$, $-\text{OCH}_3$, $-\text{NH}_2$, or
 $(\text{CH}_3)_2\text{N-}$.

In even more particularly preferred
embodiments, R_3 is selected from the group consisting of
20 amino, azido, and guanidino.

Most preferably the compound is selected from
the group consisting of

Sodium 5-acetamido-4-azido-2,3,4,5-tetradexy-D-glycero-D-galacto-non-2-enopyranosonate,
sodium 5-acetamido-4-amino-2,3,4,5-tetradexy-D-glycero-D-galacto-non-2-enopyranosonate, and
5 ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradexy-D-glycero-D-galacto-non-2-enopyranosate.

According to a second aspect, the invention provides a pharmacologically active composition comprising

- 10 (i) an orthomyxovirus or paramyxovirus-inhibiting amount of a substance that binds the active site of influenza virus neuraminidase such that said substance displays anti-orthomyxovirus or paramyxovirus activity in an animal and
15 (ii) a physiologically-compatible carrier diluent or excipient for said substance.

Preferably the substance is a compound according to general formula I, Ia, or II, but to which the provisos set out above do not apply. More preferably
20 the substance is present at a concentration of 0.000001 to 100 mg/ml.

According to a third aspect, the invention provides a method of preventing or ameliorating the symptoms of an orthomyxovirus or paramyxovirus infection,
25 comprising the step of administering to an animal a virus-inhibiting amount of a substance that binds the active site of influenza virus neuraminidase such that said substance displays anti-orthomyxovirus or paramyxovirus activity in an animal.

30 In each of these three aspects of the invention, the virus is preferably selected from the group consisting of influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus.

In the method according to the third aspect of the invention, more preferably either the virus is selected from the group consisting of influenza virus, parainfluenza virus, Sendai virus and mumps virus, and the animal is a human, or the virus is Newcastle disease virus or fowl plague virus, and the animal is a bird.

The substance may be administered orally, intranasally, buccally, or sublingually.

Preferably the substance is administered at a dose of 0.0001 to 1000 mg/kg body weight. The substance is preferably as defined above.

Brief Description of the Drawings

Figures 1 and 2 represent reaction schemes for the preparation of sodium 5-acetamido-4-azido-2,3,4,5-tetradecoxy-D-glycero-D-galacto-non-2-enopyranosonate and, the preparation of sodium 5-acetamido-4-amino-2,3,4,5-tetradecoxy-D-glycero-D-galacto-non-2-enopyranosonate respectively.

Detailed Description of the Preferred Embodiments

In general, the design of a molecule possessing stereochemical complementarity can be accomplished by means of techniques that optimize, either chemically or geometrically, the "fit" between a molecule and a target receptor. Known techniques of this sort are reviewed by Sheridan and Venkataraghavan (Acc. Chem Res. 1987, 20, 322), Goodford (J. Med. Chem. 1984, 27, 557), Beddell (Chem. Soc. Reviews 1985, 279) and Hol (Angew. Chem. 1986, 25, 767), the respective contents of which are hereby incorporated by reference. See also Blundell et al, Nature 1987, 326, 347 (drug development based on information regarding receptor structure).

The use of a refined view of the three-dimensional structure of the active site of influenza virus neuraminidase, which we have developed

(with errors of only about 0.4 Å) is described in detail in our U.S. Patent Application No. 423112 filed 19th October 1990, the entire contents of which are herein incorporated by reference. This refined structure enables the production of molecules which tightly bind the enzyme active site, something that heretofore could not have been accomplished based, for example, on extant information regarding the crystal structure on N2 influenza virus neuraminidase soaked with neuraminic acid. See Varghese et al., Nature 303: 35-40 (1983). Notwithstanding contrary expectations, discussed above, as to the import of neuraminidase-binding capability, it has also been discovered that compounds possessing high affinity for the enzyme active site are also prime candidates for in vivo anti-viral agents, which property is routinely ascertainable by means of a conventional animal assay, as described in greater detail below.

Thus, there are two preferred approaches to designing a molecule, according to the present invention, that complements the active site of influenza virus neuraminidase. By the geometric approach, the number of internal degrees of freedom (and the corresponding local minima in the molecular conformation space) is reduced by considering only the geometric (hard-sphere) interactions of two rigid bodies, where one body (the active site) contains "pockets" or "grooves" that form binding sites for the second body (the complementing molecule, as ligand). The second preferred approach entails an assessment of the interaction of respective chemical groups ("probes") with the active site at sample positions within and around the site, resulting in an array of energy values from which three-dimensional contour surfaces at selected energy levels can be generated.

The geometric approach is illustrated by Kuntz et al (J. Mol. Biol. 1982, 161, 269), the contents of which are hereby incorporated by reference, whose algorithm for ligand design is implemented in a commercial software package distributed by the Regents of the University of California and further described in a document, provided by the distributor, which is entitled "Overview of the DOCK Package, Version 1.0.", the contents of which are hereby incorporated by reference.

10 Pursuant to the Kuntz algorithm, the shape of the cavity represented by the neuraminidase active site is defined as a series of overlapping spheres of different radii. One or more extant data bases of crystallographic data, such as the Cambridge Structural Database System

15 maintained by Cambridge University, (University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.) and the Protein Data Bank maintained by Brookhaven National Laboratory (Chemistry Dept. Upton, NY 11973, U.S.A.), is then searched for molecules which approximate the shape

20 thus defined.

Molecules identified in this way, on the basis of geometric parameters, can then be modified to satisfy criteria associated with chemical complementarity, such as hydrogen bonding, ionic interactions and Van der Waals

25 interactions. The chemical-probe approach to ligand design is described, for example, by Goodford (J. Med. Chem. 1985, 28, 849), the contents of which are hereby incorporated by reference, and is implemented in several commercial software packages, such as GRID (product of

30 Molecular Discovery Ltd., West Way House, Elms Parade, Oxford OX2 9LL, U.K.) Pursuant to this approach, the chemical prerequisites for a site-complementing molecule are identified at the outset, by probing the active site (as represented via the atomic coordinates shown in Fig.

35 1) with different chemical probes, e.g., water, a methyl group, an amine nitrogen, a carboxyl oxygen, and a

hydroxyl. Favoured sites for interaction between the active site and each probe are thus determined, and from the resulting three-dimensional pattern of such sites a putative complementary molecule can be generated.

5 The chemical-probe approach is especially useful in defining variants of a molecule known to bind the target receptor. Since sialic acid is such a molecule, vis-a-vis the neuraminidase active site, crystallographic analysis of sialic acid bound to
10 neuraminidase provides useful information regarding the interaction between an archetype ligand and the active site of interest. In particular, it has been found that sialic acid binds to neuraminidase in a distorted conformation, with the carboxylate group pushed into the
15 plane of the sugar.

 Since this carboxylate-planar feature is inherent in the DANA molecule and molecules that are "DANA-like" by virtue of having an sp^2 -hybridized system at C_2/C_3 , no distortion is needed for such molecules to
20 fit - that is, to possess stereochemical complementarity with relation to - the active site. The resulting increased complementarity of DANA and DANA-like molecules is reflected, for example, in respective K_i values for DANA and a novel DANA derivative, 4-amino-2,3-dehydro-
25 2,4-dideoxy-N-acetylneuraminic acid, that are significantly lower (indicating higher active-site affinity) than the corresponding values for sialic acid and its derivatives. As described in greater detail below, the increased complementarity is also evidenced by
30 in vivo anti-viral activity of both DANA and the 4-amino DANA derivative, which was designed, according to the present invention, via the chemical-probe approach discussed above.

 Accordingly, a preferred subgroup of anti-viral
35 agents suitably used in pharmaceutical formulations of the present invention includes DANA-like molecules.

especially those with a K_1 of greater than 10^{-7} . More generally, 5-, 6- and 7-membered carbocyclic and heterocyclic compounds that possess the structural feature of carboxylate-planarity are preferred candidates for anti-viral agents to use in accordance with the present invention. Exemplary of such compounds are the molecules represented, respectively, by formula I and formula Ia. These molecules comprise a carboxylate moiety that is positioned in the plane of the ring nucleus by virtue of the sp^2 -hybridized system which includes the heteroatom or C_3 , as the case may be, and the carbon that bears the carboxylic-acid moiety or an analogue thereof, where "analogue" denotes a moiety that can interact either ionically (say, via hydrogen-bonding) or covalently (via a Schiff reaction, for instance) with a reactable amino moiety in the active site, such as is presented by arginine 371 corresponding to the coordinates for the atoms ARG NH1 371 and ARG NH2 371 of the neuraminidase three-dimensional structure disclosed in U.S. Patent Application No. 423112 referred to above.

It is known that single amino-acid changes can cause major changes in activity of influenza virus neuraminidase which are not predictable on the basis of any theoretical method. Insofar as it may not be necessary, for the complementarity between compound and active site to extend over all residues of the active site, compounds that bind atoms comprising fewer than all of the residues of the active site are encompassed by the present invention.

In summary, the general principles of receptor-based drug design can be applied by persons skilled in the art, using the crystallographic data presented above, to produce compounds having sufficient stereochemical complementarity to produce a high-affinity binding of the active site of influenza virus neuraminidase.

The present invention is further described below by reference to the following, non-limiting examples.

5 Example 1 The preparation of Sodium 5-Acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4)(4-Azido-Neu5Ac2en)

Designations of compounds are as in Figure 1.

Preparation of (2)

10 To an agitated solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (1)(1500 mg, 3.17 mmol) in a mixture of benzene (50 ml) and methanol (300 mg) was added dropwise $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (12 ml) over thirty minutes under
15 a nitrogen atmosphere at room temperature. The whole mixture was then allowed to stir at room temperature for 16 hours. The solution was diluted with ethyl acetate (250 ml), washed successively with saturated NaHCO_3 solution (30 ml x 3) and water (20 ml x 3), then

20 evaporated to a small volume (about 10 ml), to which was added water (0.5 ml) and acetic acid (0.5 ml). The whole mixture was then stirred at room temperature for two days before being diluted with ethyl acetate (200 ml). The ethyl acetate solution was washed with 5% NaHCO_3 solution
25 (30 ml x 2) and water (20 ml x 3), then evaporated to dryness. The residue was chromatographed (silica gel, ethyl acetate as eluting solvent) to afford pure compound (2) (550 mg, 40%).

$^1\text{H-NMR}$ (CDCl_3) δ (ppm): 1.95, 2.06, 2.08, 2.10, 2.35 (s, 30 15H, Acetyl CH_3 x 5), 3.80 (s, 3H, COOCH_3), 4.1-4.4 (m, 4H, H_4 , H_5 , H_6 , H_9), 4.82 (dd, 1H, $J_{9,8}$ 1.8Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.27 (m, 1H, H_8), 5.45 (bd, 1H, $J_{7,8}$ 3.5Hz, H_7), 6.15 (d, 1H, $J_{3,4}$ 5.4Hz, H_3), 6.47 (d, 1H, $J_{\text{NH},5}$ 3.8Hz, -CONH).

Preparation of (3)

To a stirred solution of compound (2) (800 mg, 1.67 mmol) in anhydrous dichloromethane (10 ml) and dry pyridine (316 mg, 4 mmol) at -30 to -40°C , was added dropwise a solution of trifluoromethane sulphonic anhydride (Trf_2O) (556 mg, 2 mmole) in dichloromethane (2 ml) over 15 minutes. The reaction mixture was then stirred at -30°C for 5 hours, and concentrated to dryness in vacuo. The residue was then dissolved in dry DMF (5 ml) containing a mixture of sodium azide (650 mg, 10 mmol) and tetrabutylammonium hydrogen sulphate (170 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 16 hours, and then evaporated to dryness under high vacuum. The residue was partitioned between ethyl acetate (200 ml) and water (50 ml). The organic layer was separated and washed with water (50 ml x 2), dried over Na_2SO_4 , evaporated to leave a residue (780 mg), which was subjected to double chromatography (silica gel, the first solvent system was ethyl acetate/acetone: 8/1; the second solvent system was dichloromethane/water: 10/1) to afford a colourless oil (3) (185 mg, 24%).

MS. (FAB) 457 ($\text{M}^+ + 1$), 414 ($\text{M}^+ - \text{N}_3$). $[\alpha]_{\text{D}}^{20} + 19.1^{\circ}$ (Cl, MeOH). i.r. (CHCl_3) cm^{-1} 2100 ($-\text{N}_3$). 1748 (carbonyl). ^1H -nmr (CDCl_3) δ (ppm). 2.04, 2.05, 2.06, 2.12, (s, 12H, Acetyl CH_3 x 4). 3.79 (s, 3H, COOCH_3), 3.91 (ddd, 1H, $\text{J}_{5,\text{NH}}$ 8.4Hz, $\text{J}_{5,4}$ 8.8Hz, $\text{J}_{5,6}$ 9.9Hz, H_5), 4.17 (dd, 1H, $\text{J}_{9,8}$ 6.8Hz, $\text{J}_{9,9}$ 12.5Hz, H_9), 4.42 (dd, 1H, $\text{J}_{4,3}$ 2.9Hz, $\text{J}_{4,5}$ 8.8Hz, H_4), 4.48 (dd, 1H, $\text{J}_{6,7}$ 2.3Hz, $\text{J}_{6,5}$ 9.9Hz, H_6), 4.64 (dd, 1H, $\text{J}_{9,8}$ 2.7Hz, $\text{J}_{9,9}$ 12.5Hz, H_9), 5.31 (m, 1H, $\text{J}_{8,7}$ 5.2Hz, $\text{J}_{8,9}$ 2.7Hz, $\text{J}_{8,9}$ 6.8Hz, H_8), 5.45 (dd, 1H, $\text{J}_{7,6}$ 2.3Hz, $\text{J}_{7,8}$ 5.2Hz, H_7), 5.96 (d, 1H, $\text{J}_{3,4}$ 2.9Hz, H_3), 6.13 (d, 1H, $\text{J}_{\text{NH},5}$ 8.4Hz, $-\text{CONH}$)

^{13}C -nmr (CDCl_3) δ (ppm)

20.7 ($\text{CH}_3\text{-CO-O-}$), 23.2 ($\text{CH}_3\text{CO-NH}$), 48.3 (C_5), 52.6 (COOCH_3), 57.8 (C_4), 62.1 (C_9), 67.7, 70.9 (C_7 , C_8), 75.9 (C_6), 107.6 (C_3), 145.1 (C_2), 161.5 (C_1), 170.2, 180.3, 170.7, (acetyl $-\text{C} = \text{O} \times 4$).

Preparation of (4)

Compound (3) (50 mg, 0.11 mmol) was dissolved in anhydrous methanol (5 ml) containing sodium methoxide (8 mg, 0.15 mmol). The mixture was stirred at room temperature for 2 hours and concentrated to dryness in vacuo. The residue was taken up in water (3 ml), stirred at room temperature for 1.5 hours, adjusted to pH 6-7 with Dowex 50 x 8 (H^+) resin, and then lyophilised to afford the title compound (4) (35 mg, 94%).

15 i.r. (KBr) cm^{-1} 3400 (br.-OH), 2100 ($-\text{N}_3$), 1714 (carbonyl).

^1H -nmr (D_2O) δ (ppm). 2.06 (s, 3H, acetyl CH_3), 3.64 (dd, 1H, $\text{J}_{9,8} 6.3\text{Hz}$, $\text{J}_{9,9} 11.8\text{Hz}$, H_9), 3.65 (dd, 1H, $\text{J}_{7,6} 3.9\text{Hz}$, $\text{J}_{7,8} 6.8\text{Hz}$, H_7), 3.88 (dd, 1H, $\text{J}_{9,8} 2.6\text{Hz}$, $\text{J}_{9,9} 11.8\text{Hz}$, H_9), 3.94 (m, 1H, $\text{J}_{8,7} 6.8\text{Hz}$, $\text{J}_{8,9} 2.6\text{Hz}$, $\text{J}_{8,9} 6.3\text{Hz}$, H_8), 4.21 (dd, 1H, $\text{J}_{5,4} 10.4\text{Hz}$, $\text{J}_{5,6} 8.9\text{Hz}$, H_5), 4.31 (dd, 1H, $\text{J}_{4,3} 2.2\text{Hz}$, $\text{J}_{4,5} 2.2\text{Hz}$, $\text{J}_{4,5} 10.4\text{Hz}$, H_4), 4.34 (dd, 1H, $\text{J}_{6,5} 8.9\text{Hz}$, $\text{J}_{6,7} 3.9\text{Hz}$, H_6), 5.82 (d, 1H, $\text{J}_{3,4} 2.2\text{Hz}$, H_3).

25 Example 2 The preparation of Sodium 5-Acetamido-4-amino-2,3,4,5-tetra-deoxy-D-glycero-D-galacto-non-2-enopyranosonate (5) (4-amino-Neu5Ac2en)

Designations of compounds are as in Figure 2.

30 Preparation of (5)

Into a solution of methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetra-deoxy-D-glycero-D-galacto-non-2-enopyranosonate (3) (95 mg, 0.208 mmol) in pyridine (6 ml) was bubbled H_2S for 16

hours at room temperature. The solution was then flushed with nitrogen for 15 minutes, and evaporated to remove pyridine under high vacuum. The residue was chromatographed (silica gel, ethyl acetate/iso-propanol/5 water = 5/2/1) to afford a colourless compound (5) (50 mg, 56%).

MS. (FAB) 431 ($M^+ + 1$), 414 ($M^+ - NH_2$), $[\alpha]_D^{20} + 34.5^\circ$ (Cl, MeOH). i.r. ($CHCl_3$) cm^{-1} 3400 (br. NH_2), 1740 (carbonyl).

10 1H -nmr ($CDCl_3 + CD_3OD$) δ (ppm). 1.96, 2.06, 2.07, 2.10 (s, 12H acetyl $CH_3 \times 4$), 3.81 (s, 3H, $-COOCH_3$), 3.92 (brt, 1H, $J_{5,4}$ & $J_{5,6}$, 10Hz, H_5), 4.17 (dd, 1H, $J_{9,8}$ 7.2Hz, $J_{9,9}$ 12.3Hz, H_9), 4.22 (br. dd, 2H, $J_{4,5}$ & $J_{6,5}$ 10Hz, $J_{4,3}$ & $J_{6,7}$ 2.1Hz, H_4 & H_6), 4.71 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9}$ 12.3Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 4.9Hz, $J_{8,9}$ 2.6Hz, $J_{8,9}$ 7.2Hz, H_8), 5.45 (d, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.9Hz, H_7), 5.97 (d, 1H, $J_{3,4}$ 2.1Hz, H_3).

^{13}C -nmr ($CDCl_3 + CD_3OD$) δ (ppm)

20.2, 20.3 ($CH_3-CO-O-$), 22.3 ($CH_3-CO-NH$), 48.2(C_5),
20 50.4(C_4), 52.0 ($COOCH_3$), 62.1(C_9), 67.8, 71.2 (C_7 , C_8), 76.5(C_6), 112.5(C_3), 143.6(C_2), 162.0(C_1), 170.2, 170.4, 170.8, 172.2. (acetyl $-C = O \times 4$).

Preparation of (6)

Compound (5) (50 mg, 0.116 mmol) was dissolved
25 in anhydrous methanol (5 ml) containing sodium methoxide (12.4 mg, 0.23 mmol). The mixture was stirred at room temperature for 1.5 hours and evaporated to dryness in vacuo at $30^\circ C$. The residue was stirred in water (3 ml) at room temperature until TLC (silica gel, ethyl
30 acetate/methanol/0.1 N HCl = 5/4/1) indicated that hydrolysis was complete. The solution (pH - about 10.5) was then gradually adjusted to around pH 7.5 by Dowex 50 x 8 (H^+) resin. As soon as the pH of the solution

reached 7.5, the suspension was quickly filtered by a press filter. The filtrate was lyophilised to afford the title compound (6) (30 mg, 83%).

^1H -nmr (D_2O) δ (ppm). 2.07 (s, 3H, acetyl CH_3), 3.59 - 3.70 m, 2H, H_7 & H_9), 3.89 (dd, 1H $\text{J}_{9,8}$ 2.6Hz, $\text{J}_{9,9}$ 11.8Hz, H_9), 3.95 (m, 1H, H_8), 3.99 (brd, 1H, $\text{J}_{4,5}$ 10.6Hz, H_4), 4.21 (brt, 1H, $\text{J}_{5,4}$ & $\text{J}_{5,6}$ 10.6Hz, H_5), 4.29 (brd, 1H, $\text{J}_{6,5}$ 10.6Hz, H_6), 5.66 (d, 1H $\text{J}_{3,4}$ 1.9Hz, H_3).

10 Example 3 Inhibition of influenza virus neuraminidase

In vitro bioassay of compounds against N2

influenza virus neuraminidase followed the same protocol as that developed by Warner and O'Brien (Biochemistry, 1979 18 2783-2787). For comparison, using the same assay

15 it was found that K_i for 2-deoxy-N-acetyl- α -D-neuraminic acid was 3×10^{-4} M.

K_i values were determined using a spectrofluorometric technique using the fluorogenic substrate ~~4-methylumbelliferyl N-acetylneuraminic acid~~

20 (MUN) described by Meyers et al. (Anal. Biochem. 101, 166-174 (1980)). For both enzymes, the assay mixture contained test compound at several concentrations between 0 and 2 mM, and approximately 1 mU enzyme in buffer (32.5 mM MES, 4 mM CaCl_2 , pH 6.5 for N2; 32.5mM Acetate, 4 mM CaCl_2 , pH 5.5 for V. cholerae neuraminidase). The reaction was started by the addition of MUN to final concentrations of 75 or 40 μM . After 5 minutes at 37°C , 2.4 ml 0.1 M Glycine-NaOH, pH 10.2 was added to 0.1 ml reaction mixture to terminate the reaction. Fluorescence
30 was read at excitation 365 nm; emission 450 nm, and appropriate MUN blanks (containing no enzyme) were subtracted from readings. The K_i was estimated by Dixon plots (1/Fluorescence versus Compound concentration). Results are summarized in Table 2.

Table 2

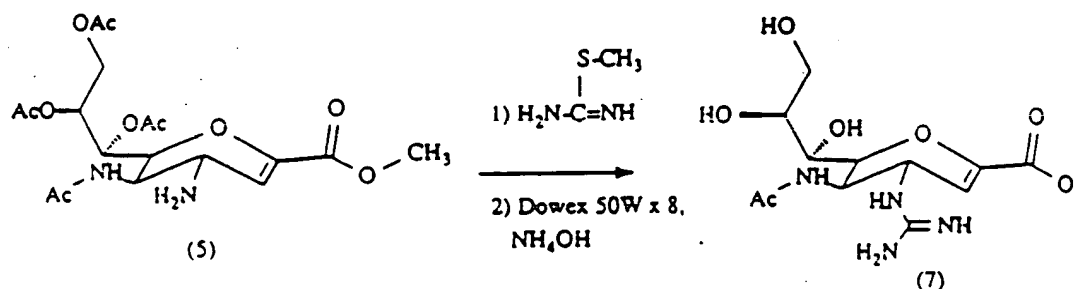
Inhibition of influenza virus neuraminidase in vitro

Compounds	K_i (M)
2-deoxy-N-acetyl- α -D-neuraminic acid	3×10^{-4}
5 Sodium 5-acetamido-4-azido-2,3,4,5-tetradecoxy- D- <u>glycero</u> -D- <u>galacto</u> -non-2-enopyranosonate	2.0×10^{-6}
Sodium 5-acetamido-4-amino-2,3,4,5-tetradecoxy- D- <u>glycero</u> -D- <u>galacto</u> -non-2-enopyranosonate	4.0×10^{-8} 1.90×10^{-7}
	(N9 neuraminidase, pH 6.5)
10	1.00×10^{-8} (N2 virus, pH 7.5)

Results are for N2 neuraminidase at pH 6.5, unless
15 otherwise indicated.

N2 virus: detergent-inactivated whole virus.

Example 4 Preparation and activity of
Ammonium 5-acetamido-4-guanidino-2,3,4,5-
tetra-deoxy-D-glycero-D-galacto-non-2-
enopyranosate (7)



5 Methyl 5,7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetra-deoxy-D-glycero-D-galacto-non-2-enopyranosate (5) (40mg, 0.093mmol) was added into a solution of S-methylisourea (546mg, 3mmol) in water (15mL) at ice-bath temperature. The reaction mixture was stirred at 5°C for seven days, then poured onto a column of Dowex 50W8(H+) resin (35mL), washed with cold water (700mL), and eluted with 1.5M NH₄OH solution. The eluate (120mL) was concentrated to dryness under high vacuum. The residue was then chromatographed (silica gel, solvent system 1:ethyl acetate/isopropanol/water, 1/5/1; solvent system 2:75% isopropanol) to afford the title compound (7) (8mg, 24.5%).

¹H-nmr(D₂O+CD₃OD) δ (ppm).
2.06(s, 2H, acetyl CH₃), 3.60(br.d., 1H, J_{7,8} 9.4 Hz, H₇), 3.63(dd, 1H, J_{9,8} 2.6 Hz, J_{9,9} 11.8 Hz, H₉), 3.76
20 (br.d., 1H, J_{4,5} 9.4 Hz, H₄), 3.87 (dd, 1H, J_{9,8} 2.6 Hz, J_{9,9} 11.8 Hz, H₉), 3.93 (ddd, 1H, J_{8,7} 9.4 Hz, J_{8,9} 2.6

Hz, J_{8,9}, 6.2 Hz, H₈), 4.01 (dd, 1H, J_{5,4} 9.4 Hz, J_{5,6} 10.6 Hz, H₅), 4.20 (br.d., 1H, J_{6,5} 10.6 Hz, H₆), 5.63 (d, 1H, J_{3,4} 2.1 Hz, H₃).

A strong positive Sakaguchi reaction was given by compound (7), indicating the presence of a guanidine group. Compound (7) was found to be a competitive inhibitor of influenza virus neuraminidase and of other neuraminidases. Results obtained using the in vitro assay described in Example 3 above are presented in Table 3.

TABLE 3

K _i (M)	Neuraminidase
1.7 x 10 ⁻⁸ (at pH 6.5)	N2 virus
6 x 10 ⁻⁹ (at pH 7.5)	N2
15 ≥ 5 x 10 ⁻⁸ (at pH 6.5)	N9
4.5 x 10 ⁻⁴ (at pH 5.8)	<u>Vibrio cholerae</u>
> 10 ⁻² (at pH 4.5)	Sheep (partially purified liver extract)

Example 5 In vivo anti-viral activity

The 4-amino compound of Example 2, which was shown in Example 4 to have anti-neuraminidase activity in vitro, was tested for anti-viral activity in vivo, using a standard type of assay.

When administered intranasally to mice before and during challenge with influenza A virus, this compound reduced the titre of virus in lung tissue 1 to 3 days after infection.

Mice were infected intranasally with 50 µl of 10³ TCID₅₀ units/mouse of H2N2 influenza A virus (A/Sing/1/57). The compound was administered intranasally at a dose rate of either 25 mg/kg body weight or 100 mg/kg body weight (50 µl of aqueous

solution/mouse) as follows: 24 hours and 3 hours before infection; 3 hours after infection; then twice daily on each of days 1, 2 and 3 after infection.

The mice were sacrificed on days 1, 2 and 3 after infection, their lungs removed and virus titres in the lungs measured. The titres were plotted graphically and expressed as the areas under the curves (AUC).

The compound showed similar potency to DANA when given intranasally to mice at a single dose level of 25 mg/kg body weight,

Pharmaceutical Compositions

A pharmaceutical formulation within the present invention combines, with an active agent that binds the viral neuraminidase active site and displays in vivo anti-viral activity, a carrier for the active agent which is pharmaceutically acceptable. A pharmaceutically acceptable carrier is a solid, liquid or gaseous material that can be used as a vehicle for administering a medicament because the material is inert or otherwise medically acceptable, as well as compatible with the active agent, in a particular context of administration. In addition to a suitable excipient, a pharmaceutically acceptable carrier can contain conventional additives like diluents, adjuvants, antioxidants, dispersing agents and emulsifiers, anti-foaming agents, flavour correctants, preservatives, solubilizing agents and colourants.

The nature of the excipient used with an anti-viral agent, pursuant to the present invention, is largely a function of the chosen route of administration, as discussed, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES, E.W. Martin (ed.), and in PHARMACEUTICAL DOSAGE FORMS AND THEIR USE, H. Hess (ed.) (Hans Huber Publ. 1985), the respective contents of which are hereby incorporated by reference. Preferably, the pharmaceutical compositions of the present invention are

provided in a unitary-dosage form which is suitable for administration intranasally, orally, buccally or sublingually.

In accordance with the present invention, a pharmaceutical composition is advantageously delivered to the throat, nasal cavity or lungs, the intranasal route of administration being especially preferred. Delivery of an active agent to the nasal cavity can be achieved with preparations of the present invention that take the form, for example, of an aerosol or vapour, a nasal spray or nose drops, or an inhalation powder. For these applications, it may be appropriate for the active agent to be micronized, for example, to a particle size on the order of 5 microns or less.

Suitable means for effecting delivery by direct application to the mucosal lining or via inhalation are well known to the art, for example, in the context of treating asthma. In this category are squeeze-bottle devices (nebulisers) and pressurized packs, for delivering a solution of the active agent as a spray into the nose, and conventional insufflators like the Spinhaler turbo-inhaler and liquid aerosol "puffers" (Spinhaler is a registered trade mark of Fisons Corporation), which deliver metered doses of a pharmaceutical preparation.

If the active agent is delivered from solution, as would typically be the case for a nasal spray or nose drops, the carrier preferably comprises distilled water that is both sterile and substantially free of fever-inducing (pyrogenic) substances, thereby to minimise the incidence of medical complications relating to contamination. Suitable propellants to comprise carriers for use in administration by pressurized aerosol are well known, including halogenated fluorocarbon gases, carbon dioxide, and nitrogen. See, e.g., Lachman et al. in THE THEORY AND PRACTICE OF INDUSTRIAL PHARMACY (Lea

and Febiger, Philadelphia 1976). In addition, a carrier for administration via intranasal delivery or insufflation may contain a pharmaceutically acceptable surface-active agent, such as a fatty acid like oleic acid or a detergent like Tween 80 or Span 80, in order to enhance uptake of the active agent.

Conventional forms which are favoured for oral administration include lozenges and pastilles, sublingual and buccal tablets, and oral sprays. Numerous carriers suitable for these forms are known, including solid pulverulent carriers comprising a simple sugar or corresponding alcohol (lactose, saccharose, sorbitol, mannitol, etc), a starch such as potato starch, corn starch or amylopectin, cyclodextrin, a cellulose derivative, and gelatine. Liquid carriers can also be employed to form suspensions, syrups, elixirs and solutions containing the active agent.

In formulating a pharmaceutical preparation of the present invention for oral administration, a solid carrier would typically be mixed with a lubricant, such as magnesium stearate, calcium stearate or a polyethylene glycol wax, and then compressed into tablet form. In keeping with common practice, tablets can be coated with a concentrated sugar solution which may contain components like gum arabic, gelatine, talcum and titanium dioxide. Alternatively, tablets can be coated with a lacquer dissolved in a readily volatile organic solvent.

A pharmaceutical composition within the present invention contains a virus-inhibiting amount of an active agent as described above. The optimum dosage of the active compound will vary with the particular case, and can be determined routinely in the clinical context, which may be prophylactic or therapeutic. 'Prophylactic' treatment is to be understood to mean treatment intended to prevent or retard second-cycle infection as defined below, thus preventing the establishment of the complete

clinical manifestations of the disease caused by that virus. 'Therapeutic' treatment is to be understood to mean treatment intended to alleviate the symptoms and severity of infection which is already established, by
5 disrupting release of virus particles and thus preventing further cycles of viral replication. Generally, the amount of active agent present in a pharmaceutical composition of the present invention should be sufficient to inhibit at least second-cycle infection by
10 orthomyxovirus or paramyxovirus in an animal. That is, an initial viral infection of a cell culminates in the assembly and budding of virus particles at the cell-membrane surface, which would be followed in the normal course by release of the particles and infection
15 thereby ("second-cycle infection") of other cells. A suitable amount of active agent to include in a pharmaceutical composition of the present invention would thus retard at least this second cycle of infection by virus, it is thought by inhibiting the action of
20 neuraminidase that results in release of virus particles from the membrane surface.

The composition may suitably be administered a few times daily at a dose level of about 0.0001 mg to 1000 mg per kg body weight.

25 It will be clearly understood that the invention in its general aspects is not limited to the specific details referred to hereinabove.

DATED this 11th day of February 1991

BIOTA SCIENTIFIC MANAGEMENT PTY LTD
By Their Patent Attorneys:

GRIFFITH HACK & CO
Fellows Institute of Patent
Attorneys of Australia

PATENT COOPERATION TREATY

PCT

FURNISHING OF COPIES OF PRIORITY DOCUMENTS

(PCT Rule 17.2(c))

From the INTERNATIONAL BUREAU

To:
AML Information Services
201 Westward Drive
Corte Madera
CA, 94976-0405

ETATS-UNIS D'AMERIQUE

Date of mailing 03 March 1997
(day/month/year) (03.03.97)

International publication No.
WO91/16320 PCT/AU91/00161

Addressee's file reference

Pursuant to the addressee's request of 26 February 1997

1. ☒ the International Bureau hereby transmits a copy of the following application(s), the priority of which was claimed in the international application:

country	date	number
AU	24.04.91	PJ 9800
AU	19.10.90	PK 2896
AU	11.02.91	PK 4537

An invoice for furnishing the above-mentioned copy(ies) will be sent under separate cover.

2. ☐ the International Bureau informs the addressee that the priority document(s) identified below has (have) not been received; consequently the requested copy(ies) cannot be furnished.

country	date	number
---------	------	--------

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

P. ASSEEFF

Telephone No. (41-22) 730.91.11

Form PCT/IB/353 (July 1992)